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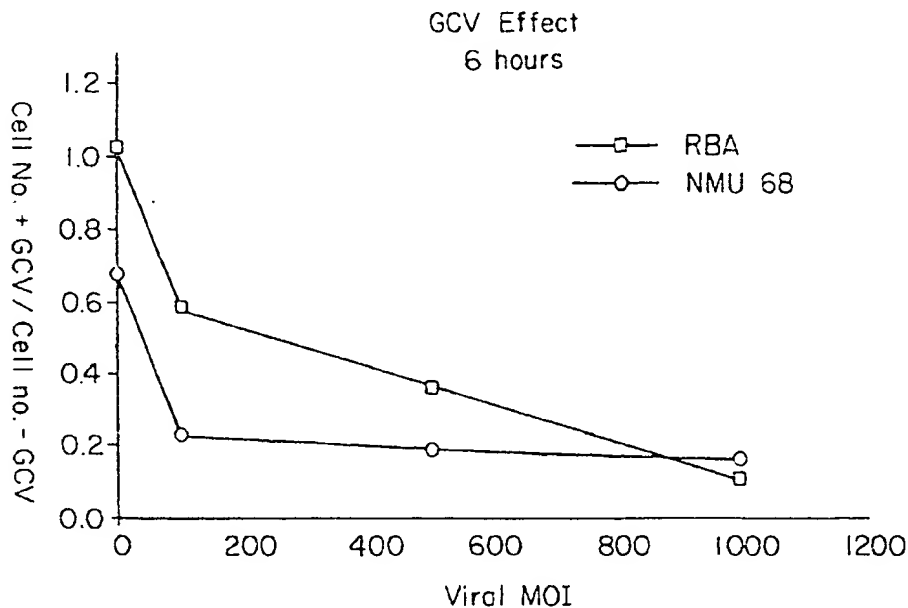
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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : A61K 38/45, 38/19, 48/00, 31/045, 35/76	A1	(11) International Publication Number: <b>WO 97/05898</b> (43) International Publication Date: 20 February 1997 (20.02.97)
(21) International Application Number: PCT/US96/12837 (22) International Filing Date: 2 August 1996 (02.08.96) (30) Priority Data: 08/510.623        3 August 1995 (03.08.95)        US (71) Applicant: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US). (72) Inventor: SUKUMAR, Saraswati, Vaidyanathan; 7283 Swan Point Way, Columbia, MD 21045 (US). (74) Agents: KILYK, John, Jr. et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 North Stetson, Chicago, IL 60601-6780 (US).	(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: DELIVERY OF AN AGENT TO THE DUCTAL EPITHELIUM IN THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF CANCER



(57) Abstract

The present invention provides prophylactic and therapeutic methods of treating the ductal epithelium of an exocrine gland, in particular a mammary gland, for disease, in particular cancer. The methods comprise contacting the ductal epithelium of the exocrine gland with an epithelium-destroying agent, preferably by ductal cannulation, so as to realize a prophylactic or therapeutic effect.

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## DELIVERY OF AN AGENT TO THE DUCTAL EPITHELIUM IN THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF CANCER

5           This application claims priority to U.S. patent  
application serial number 08/510,623, which was filed on  
August 3, 1995, and has been converted to U.S.  
provisional patent application serial number not yet  
known. The invention disclosed in this application was  
10   made with government support under NIH planning grant P20  
CA/ES66205, "Gene-mediated prevention of Cancer," and  
grant NIH 1R01 CA 57993, "Genetic and hormonal factors in  
mammary carcinogenesis," awarded by the National  
Institutes of Health, and with support of the American  
15   Cancer Society under grant RD388, "Targeted disruption of  
breast cells: a novel strategy for cancer prevention."  
Therefore, the government has certain rights in this  
invention.

20                           Technical Field of the Invention

The present invention relates to the use of an  
epithelium-destroying agent to destroy the epithelium of  
an exocrine gland, particularly the mammary gland, in the  
prophylactic and therapeutic treatment of disease, in  
25   particular cancer.

Background of the Invention

Exocrine glands are glands that release a secretion  
external to or at the surface of an organ by means of a  
30   canal or duct. Examples of exocrine glands include,  
among others, the mammary glands, prostate, liver, gall  
bladder, pancreas, kidneys, sweat glands, and salivary  
glands. Cancers of exocrine glands pose a major health  
problem, frequently resulting in death. Currently,  
35   cancers of the breast and prostate are among the leading  
causes of death among women and men, respectively.

The mature human breast comprises from six to nine major ducts, which emanate from the nipple, serially branch into ducts and terminate in lobuloalveolar structures (Russo et al., Lab. Invest. 62(3): 244-278 (1990)). This branching network of ducts is composed of epithelial cells in a supporting matrix of connective tissue and endothelial cells.

Tissues removed from the human female breast during surgery and autopsy have been examined in numerous studies directed to the nature and site of origin of neoplastic growth. Subgross sampling and histological confirmation have enabled pathological characterization of entire breasts, leading to the postulation of the existence of four major possible sites of origin of mammary carcinomas, namely ducts, terminal ducts, ductules, and acini (Russo et al., *supra*). Ductal origin is supported by the presence of more extensive epithelial proliferations, which are presumed to be preneoplastic, in surgically removed cancerous breasts as compared to nonmalignant breasts removed during autopsies (Russo et al., *supra*).

With a cumulative lifetime risk of a woman developing breast cancer estimated to be 1 in 9, there is an urgent need to develop therapeutic methods of treatment that are more effective, less invasive and accompanied by fewer side effects and prophylactic methods of treatment that are more effective than increased and intensified physical monitoring and far less extreme than radical mastectomy. In spite of the recent discovery of the heritable breast cancer susceptibility loci, BRCA1 (Miki et al., Science 266: 66-71 (1994)) and BRCA2, and other cancer susceptibility loci, and the increasing ability of physicians to identify women with elevated breast cancer risk, prophylactic methods are still currently limited to physical monitoring and prophylactic mastectomy.

In view of the above, it is an object of the present invention to provide a method of locally treating an exocrine gland prophylactically for disease. It is another object of the present invention to provide a method of locally treating an exocrine gland, in particular the mammary gland, prophylactically for cancer. Another object of the present invention is to provide a method of locally treating an exocrine gland therapeutically for disease. Yet another object of the present invention is to provide a method of locally treating a mammary gland therapeutically for cancer. Still yet another object of the present invention is to provide a method of locally treating a mammary gland both therapeutically and prophylactically for cancer. These and other objects and advantages, as well as additional inventive features, will become apparent from the detailed description provided herein.

#### Summary of the Invention

The present invention provides prophylactic and therapeutic methods of treating the ductal epithelium of an exocrine gland, in particular the mammary gland, for disease, in particular cancer. The method comprises contacting the ductal epithelium of the exocrine gland, in particular the mammary gland, with an epithelial-destroying agent. The ductal epithelium is preferably contacted with the agent by ductal cannulation. The epithelium-destroying agent is preferably a vector comprising a thymidine kinase gene, which is used in combination with ganciclovir (GCV), which can be systemically administered. Another preferred epithelium-destroying agent is a vector comprising a hypoxanthine phosphoribosyl transferase (HPRT) gene, which is used in combination with hypoxanthine aminopterin thymidine (HAT) nucleotide, which can be systemically administered. Also preferred as an epithelium-destroying agent is a vector comprising a gene, which, upon transformation of a cell

of the ductal epithelium and expression therein, induces apoptosis or death of the transformed cell. A preferred apoptosis-inducing gene is *bclxs*. Other preferred epithelial-destroying agents include a cytolytic virus, such as *Vaccinia* virus, and ethanol. The preceding methods can additionally comprise contacting the ductal epithelium with a cytokine or hematopoietic growth factor, such as GM-CSF. Also provided by the present invention is a method of treating the ductal epithelium of a mammary gland both therapeutically and prophylactically for cancer. The combined therapeutic/prophylactic method comprises treating the mammary gland therapeutically by surgery, radiation and/or chemotherapy and contacting the ductal epithelium of the mammary gland, either concomitantly or subsequently, with an epithelium-destroying agent, which does not specifically target cancerous cells. The combined therapeutic/prophylactic method can additionally comprise contacting the ductal epithelium with a cytokine or hematopoietic growth factor, such as GM-CSF.

#### Brief Description of the Figures

**Figure 1** is a graph of the ratio of cell no. in the presence of GCV over cell no. in the absence of GCV (Cell No. + GCV/Cell No. - GCV) versus viral multiplicity of infection (viral MOI), which shows the effect of the addition of 10 µg/ml GCV on NMU68 and RBA rat tumor cell lines 6 hrs after transduction with adenoviral-*Herpes simplex* thymidine kinase (AdHS-tk) at titers of 0, 100, 500 and 1,000 moi. After the cells were maintained in the presence of GCV for 3 days, they were counted using trypan blue exclusion as a measure of cell viability and cell numbers were normalized to the growth of cells in the absence of ganciclovir (GCV).

Detailed Description of the Invention

The present invention is based on the observation that the overwhelming majority of breast cancers arise from epithelial cells, particularly those epithelial  
5 cells which line the ducts of the mammary gland and are collectively referred to as the ductal epithelium. The present invention is also based on the exocrine nature of the mammary gland. Given that the mammary gland is an exocrine gland, it was further observed that the central  
10 canal or duct could provide a means of directly accessing the ductal epithelium for localized prophylactic and therapeutic treatment of cancer. Based on these observations, the prophylactic and therapeutic methods of the present invention were developed.

15

*Prophylactic Method*

The prophylactic method of the present invention is a method of treating the ductal epithelium of an exocrine gland prophylactically for a disease that affects the  
20 ductal epithelium of the exocrine gland. The method comprises contacting, preferably by ductal cannulation, the ductal epithelium of the exocrine gland with an epithelium-destroying agent so as to destroy cells of the ductal epithelium affected by the disease.

25 In one embodiment of the prophylactic method of the present invention, the ductal epithelium of a mammary gland is treated prophylactically for cancer so as to inhibit the formation of cancer of ductal epithelial origin. The method comprises contacting, preferably by  
30 ductal cannulation, the ductal epithelium of the mammary gland with an epithelium-destroying agent. The agent preferably is a vector comprising a thymidine kinase gene, such as a *Herpes simplex* thymidine kinase gene, and ganciclovir, a vector comprising a HPRT gene and HAT  
35 nucleotide, a cytolytic virus, such as a *Vaccinia* virus, or ethanol. The method can additionally comprise

contacting the ductal epithelium with a cytokine or hematopoietic growth factor, such as GM-CSF.

In another embodiment of the prophylactic method of the present invention, the ductal epithelium of an exocrine gland, such as a mammary gland, is treated prophylactically for cancer so as to inhibit the formation of cancer of ductal epithelial origin. The method comprises contacting, preferably by ductal cannulation, the ductal epithelium of the exocrine gland with an epithelium-destroying agent to destroy less than all of the ductal epithelium so as to inhibit the formation of cancer of ductal epithelial origin. Preferably, up to about 70%, 80%, 85%, 90% or 95% of the ductal epithelium is destroyed.

The epithelium-destroying agent is preferably a vector comprising a thymidine kinase gene, such as that from *Herpes simplex*, and ganciclovir, which can be brought into contact with the ductal epithelium by any suitable means, preferably by ductal cannulation or by systemic administration, a vector comprising a HPRT gene and HAT nucleotide, which can be brought into contact with the ductal epithelium by any suitable means, preferably by ductal cannulation or by systemic administration, a vector comprising a gene, which upon transformation of a cell of the ductal epithelium and expression therein, induces apoptosis or death of the transformed cell, such as *bclxs*, ethanol, or a cytolytic virus, such as *Vaccinia virus*.

The above method can additionally comprise the administration of a cytokine or hematopoietic growth factor, such as GM-CSF. The GM-CSF can be brought into contact with the ductal epithelium of the mammary gland by any suitable means, such as by ductal cannulation of GM-CSF or a vector comprising a gene encoding GM-CSF, in which case the vector can be the same vector as the one encoding the thymidine kinase, HPRT or apoptosis-inducing gene, or it can be systemically administered.



This embodiment of the prophylactic method can be used to treat any exocrine gland. However, it is particularly useful in the treatment of the mammary gland.

5       The above-described prophylactic method of treating a mammary gland is particularly useful in treating a mammary gland in a mammal at risk for developing breast cancer. The mammary gland can be characterized as one  
10       that has never had a tumor, one that had a tumor previously but the tumor is no longer detectable due to other prior therapeutic treatment, or one that has an incipient or occult tumor, preneoplasia or ductal hyperplasia. Normally, hyperplasias and incipient and  
15       occult tumors are not detectable by means of physical examination or radiography. Accordingly, the prophylactic method will find use in cases where there is reason to take some prophylactic measures, such as when there are known inherited factors predisposing to  
20       cancers, where there are suspicious lesions present in a breast with the potential for developing into a malignancy, where there has been exposure to carcinogenic agents in the environment, where age predisposes to a cancer, where cancer of another gland, e.g., the mammary gland of the contralateral breast, suggests a propensity  
25       for developing cancer, or where there is a fear or suspicion of metastasis.

#### *Therapeutic Method*

30       The therapeutic method of the present invention is a method of treating the ductal epithelium of an exocrine gland therapeutically for a disease that affects the ductal epithelium of the exocrine gland. The method comprises contacting, preferably by ductal cannulation, the ductal epithelium of the exocrine gland with an  
35       epithelium-destroying agent so as to destroy cells of the ductal epithelium affected by the disease.

In one embodiment of the therapeutic method of the present invention, the ductal epithelium of a mammary gland is locally treated therapeutically for cancer so as to destroy cancerous and noncancerous cells of the ductal epithelium and inhibit the spread of cancer. The method comprises contacting, preferably by ductal cannulation, the ductal epithelium of the mammary gland with an epithelium-destroying agent, which need not, and preferably does not, specifically target cancerous cells. The agent preferably is a vector comprising a thymidine kinase gene, such as a *Herpes simplex* thymidine kinase gene, and ganciclovir, a vector comprising a HPRT gene and HAT nucleotide, a cytolytic virus, such as a *Vaccinia* virus, or ethanol. The method can additionally comprise contacting the ductal epithelium with a cytokine or hematopoietic growth factor, such as GM-CSF.

In the therapeutic method, the epithelial-destroying agent should destroy all of the diseased or malignant epithelium. In addition, the ductal epithelium immediately surrounding the diseased/malignant epithelium also preferably should be destroyed.

#### *Combined Therapeutic/Prophylactic Method*

The present invention also provides a method of treating the ductal epithelium of a mammary gland both therapeutically and prophylactically for cancer. The method comprises treating the mammary gland therapeutically with any given therapeutic method, such as those currently known and used in the art. Examples of such methods include surgical removal of the cancerous tissue, radiation therapy and chemotherapy. The method further comprises contacting, either concomitantly with or subsequently to the therapeutic treatment, the ductal epithelium of the mammary gland, e.g., by ductal cannulation, with an epithelium-destroying agent, which preferably does not specifically target cancerous cells, so as to destroy any remaining cancerous cells and

noncancerous cells and inhibit the spread of cancer. The epithelium-destroying agent is preferably a vector comprising a thymidine kinase gene, such as a *Herpes simplex* thymidine kinase gene, combined with ganciclovir, a vector comprising a HPRT gene combined with HAT nucleotide, a cytolytic virus, such as a *Vaccinia* virus, or ethanol. The method can additionally comprise contacting the ductal epithelium with a cytokine or hematopoietic growth factor, such as GM-CSF.

#### *Alternative Embodiments of Prophylactic & Therapeutic Methods*

Although preferred embodiments have been described above, the methods of the present invention can be used to treat the ductal epithelium of any exocrine gland. Examples of exocrine glands, other than the mammary gland, which can be treated with the present inventive methods include, among others, the prostate, liver, gall bladder, pancreas, kidneys, sweat glands, and salivary glands. The methods are especially useful in the prophylactic and therapeutic treatment of the ductal epithelium of mammary glands.

Similarly, the methods can be used to treat an exocrine gland for any disease that affects the exocrine ductal epithelium. The methods are particularly useful in the treatment of cancer, including the stages of hyperplasia, adenoma, carcinoma *in situ*, and carcinoma, of ductal epithelial origin.

Any method can be used to destroy the ductal epithelium. It is preferred, however, that the destruction is limited to the ductal epithelium or a part thereof.

Any method of contacting the ductal epithelium can be used to effect local treatment. Preferably, ductal cannulation is used. Although any duct or lobule can be cannulated, it is preferred that the central canal or

duct be cannulated. Ductal cannulation also enables direct injection of a tumor mass, if desired.

Any epithelium-destroying agent can be used to destroy the ductal epithelium of an exocrine gland. The agent preferably should not destroy cells other than cells of the ductal epithelium and preferably should not result in side effects, the adversity of which outweigh the benefits of destruction of the ductal epithelium. In no event should the methods be used to destroy completely the ductal epithelium of an exocrine gland in the prophylactic/therapeutic treatment of a given disease, wherein the complete destruction of the ductal epithelium, in and of itself, would result in death of the mammal so treated.

Examples of agents that can be used in the context of the prophylactic and therapeutic methods of the present invention include cytotoxic agents. Any cytotoxic agent known in the art and suitable for contacting the ductal epithelium of an exocrine gland of a mammal can be used. In addition to ethanol and GCV described above, other examples of cytotoxic agents and their prodrugs include genistein, okadaic acid, 1- $\beta$ -D-arabinofuranosyl-cytosine, arabinofuranosyl-5-aza-cytosine, cisplatin, carboplatin, actinomycin D, asparaginase, bis-chloro-ethyl-nitroso-urea, bleomycin, chlorambucil, cyclohexyl-chloro-ethyl-nitroso-urea, cytosine arabinoside, daunomycin, etoposide, hydroxyurea, melphalan, mercaptopurine, mitomycin C, nitrogen mustard, procarbazine, teniposide, thioguanine, thiotepa, vincristine, 5-fluorouracil, 5-fluorocytosine, adriamycin, cyclophosphamide, methotrexate, vinblastine, doxorubicin, leucovorin, taxol, anti-estrogen agents such as tamoxifen, intracellular antibodies against oncogenes, the flavonol quercetin, Guan-mu-tong extract, retinoids such as fenretinide, nontoxid retinoid analogues such as N-(4-hydroxyphenyl)-retinamide (HPR), and monoterpenes such as limonene, perillyl alcohol and sobrerol.

Preferably, the agent is locally administered, especially if administration of the agent is accompanied by toxic side effects. Otherwise, the agent can be administered by any suitable route, such as systemic administration.

5       A cytolytic virus also can be used as an agent. Any cytolytic virus can be used as long as the organism mounts a rapid immunological response to it such that the virus cannot cause disease if it escapes the ductal epithelium. Examples of cytolytic viruses include  
10   Vaccinia viruses and Sindbis viruses, which can also be used as vectors. Preferably, a Vaccinia virus is used. Due to lack of mucosal immunity, Vaccinia infectious particles enter and lyse the breast epithelial cells, yet stromal immunity destroys the particles as soon as they  
15   leave the ductile tree of the exocrine gland, thereby preventing cytolysis beyond the ductal epithelium. The advantages of Vaccinia administration are that it eliminates the need for high titer virus, the need to induce cell division in the breast, and the need to  
20   administer a drug to effect cell death.

A vector comprising a suicide gene also can be used as an agent, in conjunction with an agent that destroys the ductal epithelium of an exocrine gland. The vector comprising a suicide gene, upon transformation of a cell  
25   of the ductal epithelium and expression therein, renders the transformed cell sensitive to the epithelium-destroying agent, increases the sensitivity of the transformed cell to the agent, converts the agent from a prodrug to an active drug, activates the conversion of  
30   the agent from a prodrug to an active drug, enhances the effect of the agent or, itself, produces a protein that is cytotoxic. A preferred suicide gene for use in the present inventive methods is the one described above, i.e., a thymidine kinase, such as the one from *Herpes*  
35   *simplex*, which phosphorylates GCV, which, in turn, inhibits DNA replication. Another example of a suicide gene is cytosine deaminase, which is used in conjunction

with 5-fluorocytosine. If the vector comprising the suicide gene is administered locally to the ducts, the cytotoxic agent or precursor can be administered systemically, since only transfected cells will be affected. In this regard, the bystander effect, i.e., the death of neighboring uninfected cells, presumably due to transfer of toxic byproducts through gap junctions between cells in the same compartment, obviates the need for every cell in the ductal epithelium, which is to be destroyed, to be infected. However, sufficient time must be allowed between contacting the ductal epithelium with the suicide gene and the prodrug, for example, to achieve efficient killing of the breast epithelial cells.

A vector comprising an apoptosis-inducing gene also can be used as an agent that destroys the ductal epithelium of an exocrine gland (Vaux, Cell 76: 777-779 (1994)). Examples of apoptosis-inducing genes include *ced* genes, *myc* genes (overexpressed), the *bclxs* gene, the *bax* gene, and the *bak* gene. The apoptosis-inducing gene causes death of transfected cells, i.e., by inducing programmed cell death. For example, the *bclxs* gene, *bax* gene, or *bak* gene can be used to inhibit *bcl-2* or *bcl-x<sub>L</sub>*, leading to apoptosis. Where necessary, a vector comprising an apoptosis-inducing gene can be used in combination with an agent that inactivates apoptosis inhibitors such as *bcl-2*, p35, IAP, NAIP, DAD1 and A20 proteins.

Suicide and apoptosis genes can be administered by way of a viral vector, such as an adenoviral or retroviral vector. Adenoviral vectors enable the generation of high titer recombinant viruses ( $10^{11}$ /ml) and the efficient transduction of postmitotic cells because adenoviral DNA exists as an episome in the nucleus (Verma, Molecular Medicine 1: 2-3 (1994)).

According to one preferred embodiment, the gene can be under the transcriptional regulation of a Rous sarcoma viral promoter. Alternatively, it can be under the

control of an epithelial tissue-specific or cell-specific promoter.

Uptake of recombinant virus can be facilitated by pretreatment or simultaneous treatment with polybrene or, for example, in the case of a retrovirus, attachment of the functional fragment of an antibody to the viral particle.

In another embodiment, the apoptosis gene or suicide gene can be present in a recombinant microorganism, which will express the gene. One particularly preferred microorganism for this purpose is the bacterium *Listeria monocytogenes*.

Other methods known in the art for introduction of raw DNA into cells can be used in the methods of the present invention. Alternatively, liposomes, complexes between polypeptide ligands for receptors on mammary ductal epithelial cells, including complexes of antibodies and functional fragments thereof, and plasmids can be used (Mulligan, Science 260: 926-931 (1993)). Epithelial cell-specific promoters, such as whey acidic protein (*wap*), can be used to target expression of a given gene, e.g., a suicide gene, in ductal epithelial cells. Use can also be made of wild-type tumor suppressor genes, such as p53 or Mcs-1 (rat), homeobox genes expressed in normal cells but not in cancerous cells, and the maspin gene.

Additionally, the ductal epithelium can be contacted with an agent to effect the scavenging of epithelial cells destroyed in accordance with the present invention, e.g., a cytokine/growth factor. Suitable cytokines/growth factors include GM-CSF, G-CSF, IL-2, IL-4, IL-6, IL-7, hCG, TNF- $\alpha$ , INF- $\alpha$  and INF- $\gamma$ . Such factors can be contacted with the ductal epithelium directly or by expression of a vector comprising a gene encoding the factor, in which case the vector can be the same one that comprises a suicide gene, for example. The factors stimulate a potent, long-lasting, and specific cell

immunity, requiring both CD4 and CD8 cells. The immune response is designed to scavenge destroyed ductal epithelial cells by generating autoimmunity towards epithelial cell antigens.

5       The ductal epithelium is preferably contacted with the agent by introduction of the agent through the central canal or duct of the exocrine ductal epithelium, such as by ductal cannulation. However, in the case of the mammary gland, for example, there are 6-9 major ducts  
10 that emanate from the nipple and serially branch into other ducts, terminating in lobulo-alveolar structures (Russo et al. (1990), *supra*). Accordingly, in some circumstances, such as those in which even more localized treatment is necessary or desired, for example, by the  
15 choice of anti-cancer agent, it may be preferable to contact the ductal epithelium of the exocrine gland through one of the other ducts or through a lobulo-alveolar structure as opposed to the central canal or duct. In this regard, ductal cannulation enables  
20 intratumoral injection.

The methods of the present invention can be combined with other methods of prophylactic and therapeutic treatment in addition to those cited above, such as methods that target destruction of cancer cells, e.g., by  
25 targeting of cell-surface markers, receptor ligands, e.g., ligands to gastrin-releasing peptide-like receptors, tumor-associated antigens, e.g., the 57 kD cytokeratin or the antigen recognized by the monoclonal antibody GB24, the extracellular matrix glycoprotein  
30 tamascin, antisense oncogenes such as c-fos, homeobox genes that are expressed in cancer cells but not normal cells, tumor-infiltrating lymphocytes that express cytokines, RGD-containing peptides and proteins, which are administered following surgery, lipophilic drug-  
35 containing liposomes to which are covalently conjugated monoclonal antibodies for targeting to cancer cells, low fat diet, moderate physical exercise and hormonal



modulation. For prostate cancer, anti-testosterone agents can be used as well as an inhibitor of cell proliferation produced by prostatic stromal cells and C-CAM, an epithelial cell adhesion molecule.

5       The following examples are presented to illustrate the present invention, not to limit its scope. The examples make use of the rat mammary tumor model, which has been deemed an appropriate experimental model for understanding breast cancer in humans (Sukumar et al.,  
10 Mutation Res. 333 (1-2): 37-44 (1995); Russo et al., supra). In fact, 90-100% of female rats develop mammary tumors in this model when they are administered the carcinogen NMU at 55 days of age (Sukumar, Cancer Cells 4: 199-204 (1990)).

15

#### Examples

##### *Example 1*

This example demonstrates the successful delivery of virus and other agents into the mammary ductile tree by a  
20 single injection through the teat.

ADV/CMV- $\beta$ -gal (from Dr. William Burns, Johns Hopkins University) is an adenoviral 5 vector constructed with a  $\beta$ -galactosidase gene controlled by a cytomegaloviral promoter. It was delivered into the mammary gland by  
25 injection of a viral suspension in 20  $\mu$ l of 0.2% trypan blue in Tris buffer through the teat of a rat. The nipple was extruded, and the sphincter removed by excising the nipple. In the rat, the muscle prevents fluid from regurgitating into the breast and had to be  
30 excised in order to visualize the ductal opening and administer the agent. Trypan blue was used as a tracking dye to ensure correct delivery to the ductile tree. Injection about 30 days postpartum resulted in the mammary epithelial tree being clearly visible. Ethyl  
35 alcohol (70%) was also successfully delivered by a single injection through the teat.

*Example 2*

This example demonstrates that adenovirus can efficiently transduce human mammary epithelial cells *in vitro*.

5        ADV/CMV- $\beta$ -gal was used to transduce HBL100 mammary epithelial cells *in vitro*. The  $\beta$ -gal enzyme in this construct contains a nuclear localization signal and results in dense nuclear staining. HBL100 cells ( $10^2$ ) were plated in 24-well plates, and transduced with virus  
10        at various doses and stained with X-gal 48 hrs later. Essentially all cells were infected at a moi =  $10^4$ .

      This experiment also has been performed in human mammary tumor cells MCF-7 (American Type Culture Collection (ATCC), Rockville, MD), human mammary  
15        epithelial cells MCF-10A (ATCC), and two rat mammary cancer cell lines, RBA (from Dr. Leonard Cohen) and 37-2 (from Dr. C. Marcelo Aldaz) with the same results. More efficient adenoviral constructs have been used, thereby achieving 100% infection at a moi =  $10^3$ . These  
20        experiments demonstrate successful infection by and expression of adenovirus carrying the lacZ indicator gene, which permits staining the cells blue by the expression of the enzyme  $\beta$ -galactosidase.

25        *Example 3*

This example demonstrates that infection with an AdHS-tk construct followed by GCV treatment effectively kills mammary tumor cells *in vitro*.

      RBA and NMU68 are two rat mammary tumor cell lines  
30        derived from a DMBA- and a NMU-induced tumor, respectively [DMBA = dimethylbenz[a]anthracene, NMU = N'-nitro N' -methylurea]. Each cell line was plated at a density of  $5 \times 10^2$  in 48-well plates (1.1 cm) and allowed to settle overnight. The next morning, they were  
35        transduced with AdHS-tk (Chen et al., PNAS(USA) 91: 3054-57 (1994); obtained from S. Woo and E. Aguilar-Cordova, Baylor College of Medicine, Houston, TX) at titers of 0,

100, 500, and 1000 moi, and then, 6 hrs later, GCV (10 µg/ml) was added to the culture media. The cells were maintained in the presence of GCV for 3 days and then counted using trypan blue exclusion as a measure of cell viability. The cell numbers were normalized to the growth of cells in the absence of GCV. The results are shown in Figure 1. More than 80% of the cells of each cell line were killed at a moi of  $10^3$ .

#### 10 Example 4

This example demonstrates the prophylactic effect of the method of the present invention.

The mammary glands (6 on each side) of six virgin 50 day old Sprague Dawley rats were injected with AdHS-tk on the left side and trypan blue on the right side or left untreated. One rat remained completely untreated with the virus or GCV and served as a positive control for NMU-induced tumorigenesis. On the day of surgery, rats were given an intramuscular injection of 5 µg estradiol valerate and were anesthetized with an isofluorane/O<sub>2</sub> mixture. The nipples were cannulated with a 33 gauge needle. Twenty µl of AdHS-tk diluted in trypan blue carrier (1 mM MgCl<sub>2</sub>, 20 µg/ml polybrene in 10% glycerol, 0.4% trypan blue in saline) at a concentration of  $5 \times 10^7$  particles/µl were injected into the duct. Carrier control mammary glands received 20 µl of trypan blue carrier alone. An animal was considered treated when at least three glands were successfully injected with trypan blue and another three glands were successfully injected with AdHS-tk. The remaining glands were left untreated. Twelve hrs later, the rats were injected with 125 mg/kg body weight GCV twice daily for three days. The rats were then given a second intramuscular injection of 5 µg estradiol valerate and intraperitoneal injections of 100 µg/kg body weight GCV once daily for three days. Five to seven days after GCV treatment, the rats were given an intravenous injection of NMU dissolved in 0.05% acetic

acid (Ash Stevens, CO; 50 mg NMU/kg body weight) and were subsequently monitored for general health and the appearance of tumors at weekly intervals for 8 months. The results were as follows:

5

<u>Treatment</u>	<u>No. of Tumors</u>	<u>No. of Total Glands</u>	<u>% of Glands with Tumor</u>
Untreated Control	5	12	41.7
Trypan Blue and Ganciclovir	5	17	29.4
No injection; Ganciclovir	5	20	25.0
AdHS-tk and Ganciclovir	2*	35	5.7

(\*) The two tumors (size < 5 mm) in this group were detected during necropsy at the termination of the experiment 8 months later. The difference between tumors appearing in treated versus control glands was  
10 significant by Chi<sup>2</sup> analysis (p<0.01).

The above results show that the method of the present invention inhibits the formation of cancer of ductal epithelial origin in this rat model, in which NMU  
15 induces the formation of mammary tumors in 90-100% of female rats of similar age. Surprisingly and unexpectedly, this prophylactic effect was achieved without extensive destruction of the mammary glands. These data demonstrate that selective destruction of  
20 epithelial cells, e.g., key stem cells, can be sufficient to provide prophylactic protection against carcinogen-induced tumor formation in the ductal epithelium of the mammary gland.

*Example 5*

This example demonstrates the efficient transfection  
5 of mammary epithelial cells *in vivo*.

Lytic Vaccinia virus ( $10^6$  Vaccinia-HA, which also  
carries *lacZ*, in 20  $\mu$ l 0.2% trypan blue) was injected  
into the mammary glands through the teat of 45 day old  
virgin rats. Contralateral control glands were injected  
10 with 0.2% trypan blue. The glands were excised after 3  
days. Frozen mammary gland sections were stained with X-  
gal and counterstained with eosin. When Vaccinia-HA was  
injected via the rat teat, it was able to infect the  
epithelial cells. At 3 days post-infection, the X-gal  
15 staining was confined primarily to the epithelial cells.

*Example 6*

This example demonstrates the cytotoxicity of  
20 Vaccinia/HA on HBL100 cells *in vitro*.

HBL100 cells (from ATCC, Rockville, MD) were plated  
in DME:F12 medium (50% Dulbecco's Modified Eagles Medium:  
50%Ham's F12 Supplement) containing 10% fetal bovine  
serum and 10  $\mu$ g/ml insulin at a density of  $5 \times 10^4$   
25 cells/well and incubated at 37°C overnight. Vaccinia/HA,  
at concentrations of 0 moi, 0.1 moi, or 1.0 moi, was  
added to the culture medium, and the cells were incubated  
at 37°C for at least 3 days. More than 90% of the cells  
were dead within 72 hrs of infection at 0.1 moi.

30

*Example 7*

This example shows the death of rat mammary tumor  
cells in culture by infection with Vaccinia/HA.

Cells of rat mammary cancer cell line RBA were  
35 plated in growth medium at a density of  $5 \times 10^4$  cells/well  
and incubated at 37°C overnight. Vaccinia virus  
engineered to express  $\beta$ -galactosidase and hemagglutinin

genes (*Vaccinia*/HA) was added to the culture medium at concentrations of 0 moi, 0.1 moi, or 1.0 moi and incubated at 37°C for at least 3 days. Up to 90% of the cells were lysed within 72 hours of injection at 1.0 moi.

5

#### Example 8

This example demonstrates the destruction of mammary epithelium by transfection with *Vaccinia*/HA *in vivo*.

The mammary glands of 45-day old virgin rats were  
10 injected through the teat with  $1 \times 10^7$  particles of *Vaccinia*/HA in 20  $\mu$ l 0.2% trypan blue (tracking dye). Contralateral control glands were injected with 0.2% trypan blue. The glands were excised after 3 days, fixed in chloroform:methanol:acetic acid and stained in iron-  
15 hematoxylin. Branching structures of a whole-mounted mammary gland injected with tracking dye alone were visible up to the end buds and alveoli. Also visible as brown bodies were the mammary lymph nodes. Examination of a whole-mounted mammary gland of the same rat  
20 receiving *Vaccinia*/HA in trypan blue on the contralateral side revealed that only about 30% of the ducts remained. In addition, the lymph nodes were considerably enlarged, denoting the mounting of an immune response to clear the *Vaccinia* from the vicinity.

25

All publications, patents, and patent applications cited herein are hereby incorporated by reference to the same extent as if each individual document were individually and specifically indicated to be  
30 incorporated by reference and were set forth in its entirety herein.

While this invention has been described with emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that the preferred  
35 embodiments may be varied. It is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes

all modifications encompassed within the spirit and scope  
of the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating the ductal epithelium of an exocrine gland prophylactically for cancer, which  
5 method comprises contacting the ductal epithelium of the exocrine gland with an epithelium-destroying agent to destroy less than all of the ductal epithelium so as to inhibit the formation of cancer of ductal epithelial origin.  
10
2. The method of claim 1, wherein said epithelium-destroying agent is brought into contact with the ductal epithelium by ductal cannulation.
- 15 3. The method of claim 2, wherein said exocrine gland is a mammary gland.
4. The method of claim 3, wherein up to about 90% of the ductal epithelium is destroyed.  
20
5. The method of claim 4, wherein up to about 80% of the ductal epithelium is destroyed.
6. The method of claim 5, wherein up to about 70%  
25 of the ductal epithelium is destroyed.
7. The method of claim 3, wherein essentially only the stem cells of the ductal epithelium are destroyed.
- 30 8. The method of claim 3, wherein said epithelium-destroying agent is a vector comprising a thymidine kinase gene combined with ganciclovir.
9. The method of claim 8, wherein said  
35 ganciclovir, instead of being brought into contact with the ductal epithelium by ductal cannulation, is brought



into contact with the ductal epithelium by systemic administration.

10. The method of claim 8, which additionally  
5 comprises contacting the ductal epithelium with a cytokine or hematopoietic growth factor.

11. The method of claim 10, wherein said  
10 hematopoietic growth factor is GM-CSF.

12. The method of claim 11, wherein said GM-CSF is brought into contact with the ductal epithelium by ductal cannulation of GM-CSF or a vector comprising a gene encoding GM-CSF.

13. The method of claim 12, wherein said vector comprising a gene encoding GM-CSF is the same vector comprising the thymidine kinase gene.

14. The method of claim 3, wherein said epithelium-destroying agent is a vector comprising a HPRT gene combined with HAT nucleotide.

15. The method of claim 14, wherein said HAT nucleotide, instead of being brought into contact with the ductal epithelium by ductal cannulation, is brought into contact with the ductal epithelium by systemic administration.

16. The method of claim 14, which additionally comprises contacting the ductal epithelium with a cytokine or hematopoietic growth factor.

17. The method of claim 16, wherein said  
35 hematopoietic growth factor is GM-CSF.

18. The method of claim 17, wherein said GM-CSF is brought into contact with the ductal epithelium by ductal cannulation of GM-CSF or a vector comprising a gene encoding GM-CSF.

5

19. The method of claim 18, wherein said vector comprising a gene encoding GM-CSF is the same vector comprising the HPRT gene.

10

20. The method of claim 3, wherein said epithelium-destroying agent is a vector comprising a gene, which, upon transformation of a cell of the ductal epithelium and expression therein, induces apoptosis or death of the transformed cell.

15

21. The method of claim 20, wherein said gene is *bclxs*.

20

22. The method of claim 20, which additionally comprises contacting the ductal epithelium of the mammary gland with a cytokine or hematopoietic growth factor.

25

23. The method of claim 22, wherein said hematopoietic growth factor is GM-CSF.

30

24. The method of claim 23, wherein said GM-CSF is brought into contact with the ductal epithelium by ductal cannulation of GM-CSF or a vector comprising a gene encoding GM-CSF.

25. The method of claim 24, wherein said vector comprising a gene encoding GM-CSF is the same vector comprising the apoptosis-inducing gene.

35

26. The method of claim 3, wherein said epithelium-destroying agent is ethanol.

27. The method of claim 26, which additionally comprises contacting the ductal epithelium of the mammary gland with a cytokine or hematopoietic growth factor.

5

28. The method of claim 27, wherein said hematopoietic growth factor is GM-CSF.

29. The method of claim 3, wherein said epithelium-destroying agent is a cytolytic virus.

10

30. The method of claim 29, wherein said cytolytic virus is a Vaccinia virus.

15

31. The method of claim 30, which additionally comprises contacting the ductal epithelium of the mammary gland with a cytokine or hematopoietic growth factor.

32. The method of claim 31, wherein said hematopoietic growth factor is GM-CSF.

20

33. A method of treating the ductal epithelium of a mammary gland prophylactically for cancer, which method comprises contacting, by ductal cannulation, the ductal epithelium of the mammary gland with an epithelium-destroying agent selected from the group consisting of a vector comprising a thymidine kinase gene combined with ganciclovir, a vector comprising a HPRT gene combined with HAT nucleotide, a cytolytic virus, and ethanol so as to inhibit the formation of cancer of ductal epithelial origin.

25

30

34. The method of claim 33, wherein said cytolytic virus is a Vaccinia virus.

35

35. The method of claim 33, which additionally comprises contacting the ductal epithelium with a cytokine or hematopoietic growth factor.

5        36. The method of claim 35, wherein said hematopoietic growth factor is GM-CSF.

37. A method of treating the ductal epithelium of an exocrine gland prophylactically for a disease, other  
10 than cancer, which affects the ductal epithelium of the exocrine gland, which method comprises contacting, by ductal cannulation, the ductal epithelium of the exocrine gland with an epithelium-destroying agent so as to destroy cells of the ductal epithelium affected by the  
15 disease.

38. A method of treating the ductal epithelium of a mammary gland therapeutically for cancer, which method comprises contacting, by ductal cannulation, the ductal  
20 epithelium of the mammary gland with an epithelium-destroying agent, which does not specifically target cancerous cells, so as to destroy cancerous and noncancerous cells of the ductal epithelium and inhibit the spread of cancer.

25        39. The method of claim 38, wherein said epithelium-destroying agent is selected from the group consisting of a vector comprising a thymidine kinase gene combined with ganciclovir, a vector comprising a HPRT  
30 gene combined with HAT nucleotide, a cytolytic virus, and ethanol.

40. The method of claim 39, wherein said cytolytic virus is a *Vaccinia* virus.

35

41. The method of claim 39, which additionally comprises contacting the ductal epithelium with a cytokine or hematopoietic growth factor.

5        42. The method of claim 41, wherein said hematopoietic growth factor is GM-CSF.

10        43. A method of treating the ductal epithelium of an exocrine gland therapeutically for a disease, other than cancer, which affects the ductal epithelium of the exocrine gland, which method comprises contacting, by ductal cannulation, the ductal epithelium of the exocrine gland with an epithelium-destroying agent so as to destroy cells of the ductal epithelium affected by the  
15        disease.

20        44. A method of treating the ductal epithelium of a mammary gland both therapeutically and prophylactically for cancer, which method comprises:  
      (a) treating said mammary gland therapeutically with a method selected from the group consisting of surgical removal of the cancerous tissue, radiation therapy, and/or chemotherapy; and  
      (b) contacting, either concomitantly with or  
25        subsequently to (a), the ductal epithelium of the mammary gland with an epithelium-destroying agent, which does not specifically target cancerous cells, so as to destroy any remaining cancerous cells as well as noncancerous cells and inhibit the spread of  
30        cancer.

35        45. The method of claim 44, wherein said epithelium-destroying agent is selected from the group consisting of a vector comprising a thymidine kinase gene combined with ganciclovir, a vector comprising a HPRT gene combined with HAT nucleotide, a cytolytic virus, and ethanol.

46. The method of claim 45, wherein said cytolytic virus is a *Vaccinia* virus.

5        47. The method of claim 45, which additionally comprises contacting the ductal epithelium with a cytokine or hematopoietic growth factor.

10       48. The method of claim 47, wherein said hematopoietic growth factor is GM-CSF.

49. The method of claim 44, wherein said ductal epithelium is contacted by ductal cannulation.

1/1

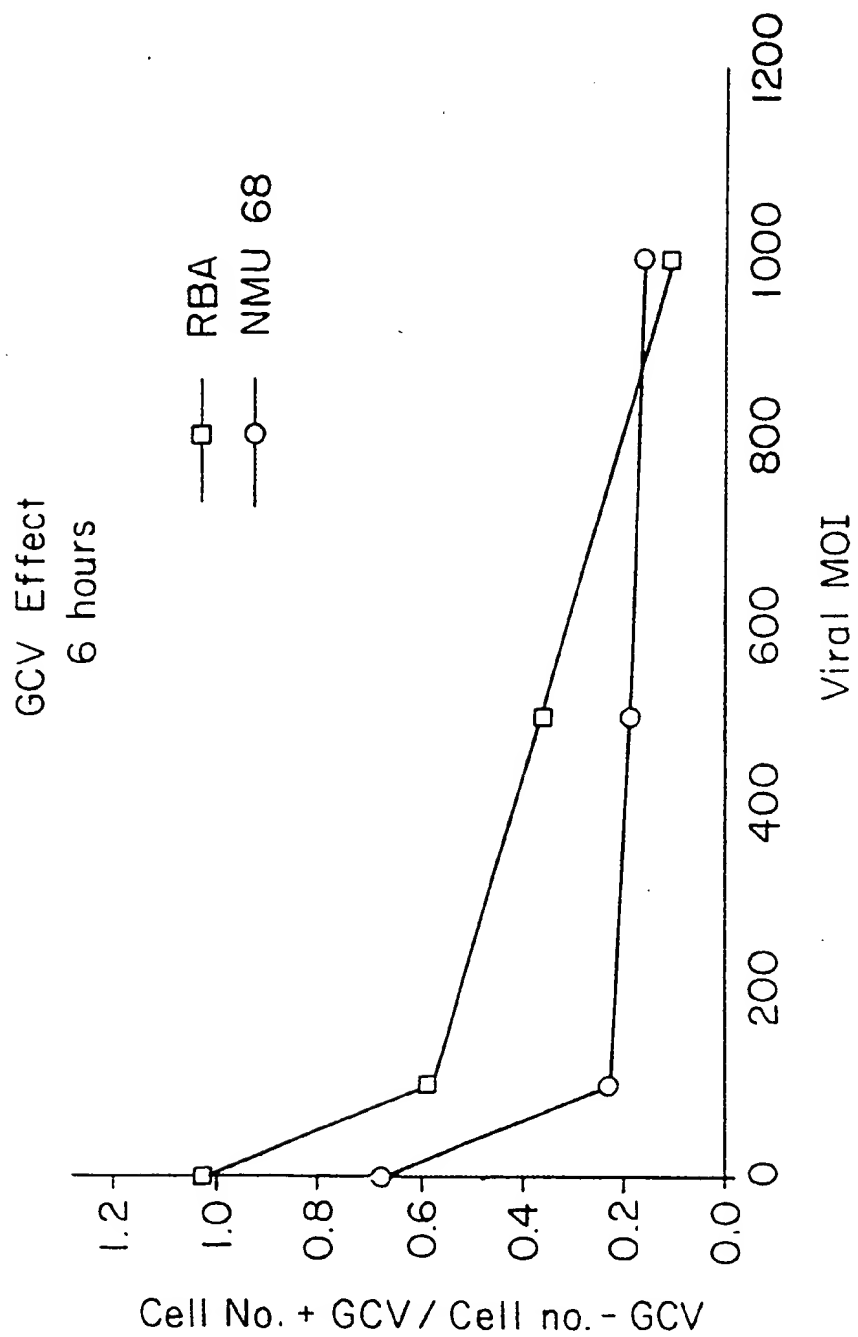


FIG. 1

# INTERNATIONAL SEARCH REPORT

International Application No  
PC1/US 96/12837

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/45 A61K38/19 A61K48/00 A61K31/045 A61K35/76

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 170, 1989, pages 1649-1669, XP000612051 TAMM ET AL: "INTERLEUKIN 6 DECREASES CELL-CELL ASSOCIATION AND INCREASES MOTILITY OF DUCTAL BREAST CARCINOMA CELLS" see the whole document ---	1-49
A	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT 94273121, FRYKBERG ET AL: "OVERVIEW OF THE BIOLOGY AND MANAGEMENT OF DUCTAL CARCINOMA IN SITU OF THE BREAST" XP002020885 & CANCER (1994 JUL 1) 74 (1 SUPPL) 350-361 see abstract --- -/--	1-49

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

11 December 1996

Date of mailing of the international search report

07. 01. 97

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCI/US 96/12837

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 118 365 (HEALTH RESEARCH INC) 12 September 1984 see page 7, line 17 - page 11, line 10 ---	1-49
A	EP,A,0 352 722 (ASPEN DIAGNOSTICS CORP) 31 January 1990 see page 4, line 38 - page 5, line 14 see page 16; table D see page 19; table E ---	1-49
A	EP,A,0 657 174 (MAYO FOUNDATION) 14 June 1995 see page 2, line 36 - line 42 ---	1-49
A	WO,A,94 26915 (UNIV MICHIGAN) 24 November 1994 see page 3, line 29 - page 4, line 34 ---	
A	WO,A,93 02556 (UNIV ROCHESTER) 18 February 1993 see page 6, line 14 - page 11, line 8 see page 14, line 34 - page 15, line 11 ---	
A	WO,A,94 09160 (JOLLA CANCER RES FOUND) 28 April 1994 see page 5, line 22 - page 7, line 23 ---	
A	LABORATORY INVESTIGATION, vol. 62, 1990, pages 244-278, XP000612052 RUSSO ET AL: "BIOLOGY OF DISEASE. COMPARATIVE STUDY OF HUMAN AND RAT MAMMARY TUMORIGENESIS" cited in the application ---	
A	VIRCHOWS ARCHIV A PATHOLOGICAL ANATOMY AND HISTOPATHOLOGY, vol. 413, 1988, pages 3-10, XP000612025 ZENKLUSEN ET AL: "THE IMMUNOHISTOCHEMICAL REACTIVITY OF A NEW ANTI-EPITHELIAL MONOCLONAL ANTIBODY (MAB B-12) AGAINST BREAST CARCINOMA AND OTHER NORMAL AND NEOPLASTIC HUMAN TISSUES" ---	
A	HYBRIDOMA, vol. 8, 1989, pages 293-302, XP000612021 TAMAKI ET AL: "A HUMAN MONOCLONAL ANTIBODY DERIVED FROM AXILLARY LYMPH NODES OF A BREAST CANCER PATIENT" -----	

# INTERNATIONAL SEARCH REPORT

International Application No

PL 1/US 96/12837

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WO-A-9409160	28-04-94	US-A- 5516631 CA-A- 2147127 EP-A- 0666929 JP-T- 8504573	14-05-96 28-04-94 16-08-95 21-05-96

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 12837

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Please see Further Information sheet enclosed.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 1 - 49 in so far as they relate to in vivo methods, of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.